Marker assisted selection for biotic stress resistance in wheat and rice

K. V. Prabhu*, A. K. Singh, S. H. Basavaraj, D. P. Cherukuri, A. Charpe, S. Gopala Krishnan, S. K. Gupta, M. Joseph, S. Koul, T. Mohapatra, J. K. Pallavi, D. Samsampour, A. Singh, Vikas K. Singh, A. Singh and V. P. Singh

Division of Genetics, Indian Agricultural Research Institute, New Delhi 110 012

Abstract

Use of molecular markers has emerged as a powerful and efficient approach to complement traditional plant breeding for improving crops. An array of molecular markers are now available that include RFLP that is based on Southern blot hybridization and, RAPD, ISSR, SSR and STS are based on polymerase chain reaction. The AFLP and CAPS markers are the other PCR based markers involving pre and post amplification restriction digestion, respectively. The most recent marker system is single nucleotide polymorphism (SNP) that utilizes the vast DNA sequence resources available in different crop species. Each of these markers has its own strengths and limitations. Markers are being used in several different aspects of crop improvement including estimation of genetic diversity, construction of high density genome maps, mapping and tagging of genes, map-based isolation of genes and marker assisted selection (MAS). MAS is carried out for transferring target gene(s) from one genetic background to another using tightly linked markers (foreground selection). MAS is also carried out to quickly recover recurrent parent genome in backcross breeding using a large number of either random or mapped markers having whole genome coverage (background selection). Hence, MAS requires markers tightly linked to the genes for the target traits as well as high-density genome maps in crops of interest. This condition is not fulfilled in all crops and traits. The Division of Genetics, IARI has taken a lead in this approach in breeding for rust resistance in wheat, blight and blast resistance in rice. MAS has been effectively employed in pyramiding identified genes involving short breeding cycles through background and foreground selection thereby adding resistance to established cultivars of each crop.

Key words : Wheat, rice, molecular marker, MAS, foreground selection, background selection

Introduction

In the last decade and half, plant breeding has adopted modern tools of biotechnology that has made the breeding processes more precise than before. Plant breeding involves creation, selection and fixation of superior genotypes with desirable traits resulting in improved varieties/ hybrids suited to the needs of consumers and farmers. But plant breeders face an endless task of continually developing new crop varieties [1], which is attributed to spontaneous but unavoidable changes in agricultural practices, evolving target environment including organisms influencing crop productivity (pests and diseases), altered growth conditions and change in consumer preferences [2]. One of the simplest tools of biotechnology is the technology that is broadly termed as "Marker Assisted Selection" (MAS). MAS is the process of selecting for a desirable expression of a character such as resistance to a disease without actually measuring the expression of the character.

Molecular markers are nothing but simple stretches of DNA in an organism located on the same chromosome in a tight linkage with the resistant gene or genes or regions on the chromosome (also referred to as quantitative trait loci or QTLs). These are linked in such a manner that if the markers linked to any particular gene for resistance are observed from the total DNA sample of the plant, then it is assured that in all probability the plant would also be resistant to the disease. Thus, this technology is free of any dependence on environment for selection of a plant. The only limiting feature is the accuracy with which the linkage between molecular markers are mapped as linked with genes for resistance to the disease.

Every disease will depend for its spread on the climatic conditions desired by the disease causing organism (pathogen), its infection load (either in air, water, soil or seed) and availability of a disease susceptible host plant. If any of the three miss out, then the disease does not occur. In the case of breeding

^{*}Corresponding author's e-mail: kvinodprabhu@rediffmail.com

Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012 Online management by indianjournals.com

disease resistant varieties of crops, plant breeders have been exploring the trait of genetic resistance to the disease available in the plant species to make a variety resistant to the disease. Crosses between the resistant donor plant and susceptible variety are affected in order to select for the transfer of the character of resistance into the susceptible variety. This is done by continuous selection for the disease resistant plants in the progeny populations of the crop. But, because of dependence on multiple factors including those which are beyond control associated with the climatic conditions or due to the lack of inoculum of the pathogen or those strains which are particularly virulent on most varieties (the strains are also known as races which can infect differentially the host resistance in different varieties), it would be impossible to be precise and sure that the selected resistant plant progenies are actually resistant because of the resistant genes in them. It is quite possible that because of the above factors, the disease would not have appeared on most plants and therefore a susceptible plant would behave like a resistant plant. Marker assisted selection eliminates this ambiguity associated with conventional environment dependent selection for disease resistance. That is because, the molecular markers have the ability to detect resistance in a plant even when the plant is not challenged by the pathogen or its races.

In recent years, molecular markers have been developed based on the more detailed knowledge of genome structure and molecular marker technology has emerged as a powerful and efficient selection tool to complement traditional plant breeding approaches for improving crops.

Marker assisted selection

Marker assisted selection (MAS) is based on the concept that it is possible to infer the presence of a gene from the presence of a marker that is tightly linked to the gene. The use of genetic markers as a reliable tool for the plant breeder was recognized by Saxway back in 1923 [3]. However, its application was largely hindered by the lack of suitable markers and nonavailability of detailed genetic linkage maps. The rapid development of molecular techniques has opened up sources of genes to plant breeding that were not available previously through conventional breeding [4]. The use of molecular markers provides an opportunity to select desirable lines based on genotypes rather than phenotypes. This approach has been extremely attractive to breeders for analyzing plants at seedling stage, screening multiple traits that would ultimately be

epistatic with one another, minimizing linkage drag and rapidly recovering the recurrent parent genotype are just few attractions of MAS in plant breeding [5].

MAS - Some essential considerations

The success of MAS in a plant breeding programme depends on three important factors namely (i) cosegregation or tight linkage of markers (≤ 5 cM) with the desired trait, (ii) an efficient, user-friendly, cost effective means to screen large populations for the molecular marker(s) and (iii) high reproducibility of the screening technique across laboratories.

Gene pyramiding

The term gene pyramiding in plant breeding is used to signify the activity of building into one variety more than one gene for a character so that the expression of the character is enhanced. It can be generally two or sometimes four genes put together in a variety. For example Sr25 and Sr26, two genes for resistance to stem rust disease in wheat. Evolution-wise, if there is one gene for resistance in a variety that is able to protect the variety against the existing strains or races of the stem rust in a region, over a period of say 4-5 years on an average, till the pathogen population throws up new genetic variants by a random process of genetic mutation that may develop the ability to overcome the resistance of the gene. But if both the Sr genes are put together in the same variety, the frequency of variants in the strain which can mutate to affect the resistance by both these genes is very low compared to the frequency if only one resistant gene were to be present. It takes that much longer for the variants of the pathogen to counter more than one gene based pyramided resistance.

Technically, each gene can be distinguished from the other when there are one or more races of the stem rust pathogen which can successfully overcome the genetic resistance of one of the two genes and another set of race/s which can overcome both the genes. But more often than not, there are quite a few Sr genes which do not necessarily have any races which can differentially react with either gene. With this lack of pathogen races which can distinguish among these Sr genes (referred to as differential races), it would be impossible to detect the presence of any of genes in combinations in breeding populations through conventional means. It is here the role of molecular markers assumes significance because, the molecular markers linked to each of these pyramided genes separately can conveniently help the breeders to pick up different combinations as desired by them in the progeny plants of the crosses between disease susceptible variety and donor lines carrying the disease resistance genes with precision without being dependent on the expression of resistance or screening for resistance.

Durable resistance

In late 1970s, a phenomenon of disease resistance response came to be noted as existing in several plant varieties which seem to show a high level of tolerance to a disease ranging from hypersensitive resistance to low severity to all races of a pathogen, even after the cultivation of the variety for a fairly long period of time in large areas. One such case was noticed by late Dr. Roy Johnson of United Kingdom in wheat against stripe rust, a feature that he termed as "durable resistance". The durable resistance as a concept is a relative capacity of a variety that has been in cultivation for a long period, expectedly beyond five years and grown to large areas at a time. The fact in terms of genetics behind this phenomenon is that either there is a quantitative resistance governed by QTLs or by a combination of specific genes, more than one in number.

Identification of Molecular Markers and MAS for rust resistance in wheat

Leaf rust resistance in wheat has been a major area of focus for plant breeders in all areas where wheat is cultivated. Use of chemicals to control the rust is expensive and environmentally hazardous, and the deployment of the resistant cultivars is the most effective method to control the leaf rust pathogen. Till date plant breeders, geneticists, plant pathologists and biotechnologists are working in sync to ensure concerted efforts to combat the invasion by this pathogen.

Economic losses vary from area to area depending on the climatic conditions and the degree and range of resistance present in cultivars. Infections can lead to 1- 20% yield loss because, the infected leaves die earlier and all the nutrients are directed to the growing fungi. Individual fields can be destroyed when the disease is severe prior to heading. Losses are often the greatest in years most favorable for wheat growth, thus, high yields and higher losses often occur together. Grain shrivels as nutrients produced primarily in the flag leaf are used by the fungus rather than transported to the grain [6].

The number of named and later mapped leaf rust (*Lr*) resistance genes in wheat increased significantly

during the last decade. A significant number of Lr resistance genes originated from Triticum aestivum cultivars, but some resistance genes were originally introgressed into common wheat cultivars from wild species accessions: Aegilops umbellulata, Aegilops squarrosa, Agropyron elongatum, Aegilops speltoides or Aegilops ventricosa. Successful translocation between wheat and the alien chromosomes was observed as a scientific breakthrough. Since it is expected that Lr genes sourced from wild relatives are likely to be more durable, several have been transferred into wheat from its wild relatives [7, 8] and many of these have been documented as located on different chromosomes [9]. At the Division of Genetics, IARI many of these genes are being selected for using available validated molecular markers or by identifying new markers which are validated for their repeated use in multiple genetic backgrounds (Table 1).

 Table 1. List of Lr genes and the associated molecular markers employed in breeding exercises at I.A.R.I.

Gene	Location	Origin	Marker	Reference	
Lr9	6BL	Triticum umbellulatum	SCAR	[10]	
Lr19	7DL	Thinopyrum ponticum	SSR	[11]	
Lr24/Sr24	3DL	Thinopyrum ponticum	SCAR	[12]	
Lr28	4AL	Triticum speltoides	SCAR	[13]	
Lr48	4BL	Triticum aestivum	RAPD	[14]	

At IARI, many genes were mapped with RAPDs and microsatellite markers and, the RAPD markers closely linked with the *Lr* genes were converted to sequence characterized amplified region (SCAR) markers (Fig. 1).

Pyramiding Lr genes in different genetic backgrounds

Gene pyramiding through conventional methods is difficult and time consuming because it requires simultaneous tests of the same wheat breeding materials with several different rust races before a selection is made. Usually, it is not feasible for a regular breeding program to maintain all necessary rust races needed for this type of work. Therefore, molecular marker assisted selection (MAS) is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust-resistant cultivars [15].



Fig. 1. Lr genes mapped and tagged with different molecular markers in wheat

Singh et al. [16] reported that the efforts to breed cereals for resistance to rust diseases have identified resistance expressed at seedling growth stage (called as seedling resistance that is effective throughout the life of the plant) and resistance that is effective on adult plants only (called adult plant resistance or APR). Most of the resistance genes confer resistance in the seedling stage itself enabling the plant to resist the invasion by the fungus during the entire growing period. The APR provides the plant an ability to withstand the extreme effect of the infecting virulence by showing hypersensitive reaction as a consequence of the ability of the fungus to infect the plant. It is acknowledged that leaf rust control could be most effective if APR is utilized in combination with seedling resistance in wheat breeding programs [17]. The most prominent example of the interactive complementary effects resulting in enhanced reaction of APR genes in the presence of seedling resistance genes is the case of durability of the resistance by the APR gene Lr34 in the presence of seedling resistance [18]. In such an attempt, three pyramided wheat lines were developed to provide for durable resistance to leaf rust. One of them is HD 2329

+ Lr9 + Lr24 + Lr28, a three-gene pyramid. Lr9, Lr24 and Lr28 are all seedling leaf rust resistance genes and are widely employed to provide for resistance to leaf rust in India (Fig. 2).

Two two-gene pyramids, PBW 343 + Lr24 + Lr48and PBW 343 + Lr28 + Lr48 were also developed in the background of PBW343, the highly cultivated wheat variety in India. Again, dominant markers RAPD and SCAR were employed to achieve pyramiding (Fig. 3). The RAPD marker pair – S3450 and S336775 linked in repulsion and coupling phase respectively, to the *Lr48* locus have been utilized as a codominant marker system.

Molecular markers and marker assisted selection in rice

An array of DNA markers namely Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Site (STS), Sequence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphisms (AFLPs), Microsatellites/Simple Sequence Repeats (SSRs), also known as Sequence Tagged Microsatellite



a.: Amplification of a 550bp band by the *Lr9* specific SCAR marker SCS5; b.: Amplification of a 607bp band by the *Lr24* specific SCAR marker SCS1302; c: Amplification of a 570bp band by the *Lr28* specific SCAR marker SCS421; M: 100bp DNA ladder; P1: HD2329 + *Lr9*; P2: HD2329 + *Lr24*; P3: HD2329 + *Lr28*

Fig. 2. Representative gels identifying the respective genes in the individual plants of the three-gene pyramided progeny in the F₅ generation



(a)







a.: Amplification of a 607bp band by the *Lr24* specific SCAR marker SCS1302; b.: Amplification of a 450bp band in the *Lr48* carrying plants by the recessive resistance allele specific RAPD marker S3₄₅₀; c: Amplification of a 775bp band in the *Lr48* carrying plants by the dominant susceptibility allele specific RAPD marker S336₇₇₅; M: 100bp DNA ladder; P1: CSP44-*Lr48*; P2: PBW343 + *Lr24* d.: Amplification of a 570bp band by the *Lr28* specific SCAR marker SCS421; e.: Amplification of a 450bp band in the *Lr48* carrying plants by the recessive resistance allele specific RAPD marker S3₄₅₀; f: Amplification of a 775bp band in the *Lr48* carrying plants by the recessive resistance allele specific RAPD marker S3₄₅₀; f: Amplification of a 775bp band in the *Lr48* carrying plants by the dominant susceptibility allele specific RAPD marker S336₇₇₅. M: 100bp DNA ladder; P1: CSP44-*Lr48*; P2: PBW343 + *Lr28*

Fig. 3. Representative gels identifying the respective genes in the individual plants of the two-gene pyramided progeny in the F₅ generation

Sites (STMS) and more recently sequencing based markers such as Single Nucleotide Polymorphisms (SNPs) have been developed in rice [19-24].

Marker Assisted Selection for incorporating/ pyramiding genes for biotic stress tolerance in rice

Important biotic stresses in rice includes diseases such as bacterial leaf blight (caused by *Xanthomonas oryzae* pv. *oryzae*), blast (caused by the fungus *Magnaporthe grisea*) and rice tungro virus; insect pests such as gall midge, brown planthopper, green leafhopper (*Nephotettix virescens*), and green rice leafhopper,

Disease	Gene	Donor parent	Recurrent parents	Chr' location	Linked marker	Linkage distance	Reference
Bacterial blight	xa13	IRBB55	Pusa Basmati 1, PRR78 and Pusa 6B	8	RG136 (CAPS)	3.7 cM	[26]
	Xa21			11	pTA248 (STS)	Gene base	d
Blast	Piz-5	C101A51	Pusa 6B PRR78& Pusa Basmati 1	6	AP5930 (STMS)	0.05 cM	[27]
	Pi-K ^h	Tetep		11	RM206 (STMS)	0.6 cM	[28]
	Pi-1	DHMAS70 Q164-2a	Pusa Basmati 1	11	RM224 (STMS)	0.0 cM	[29]
	Pi-ta	DHMAS70 Q164-2a	Pusa Basmati 1	12	RM247 (STMS)	< 5.0 cM	[30]
	Pi-b	IRBLB-b	Pusa Basmati 1	2	RM208 (STMS)	1.2 cM	[31]
	Pi-5	IRBL5-M	Pusa Basmati 1	9	S04G03 (STS)	0.8 cM	[32]
	Pi-9	IRBL-9-W	Pusa Basmati 1	6	AP5930 (STMS)	0.05 cM	[27]

 Table 2.
 The list of molecular markers validated for use in MAS for gene(s) governing resistance to bacterial blight and blast

which account for significant yield losses. Using the saturated rice molecular map and genome sequence information, a number of genes for biotic stress resistance have been isolated from the genome using a map based cloning strategy. These include bacterial blight resistance genes *Xa1*, *xa5*, *xa13*, *Xa21*, *Xa26*, and *Xa27*; blast resistance genes *Pib*, *Pita*, *Pi2*, *Pizt*, *Pi9*, *Pid2*, *Pi36*, and *Pi37* [25]. The DNA markers have been used effectively to identify resistance genes, and MAS has been applied for integrating different resistance genes into rice cultivars lacking the desired traits [22].

Validation of molecular markers linked to resistance genes for bacterial blight and blast in rice

One of the essential steps in marker assisted selection is the validation of the molecular markers linked to the gene of interest between the donor and recurrent parents. The markers reported to be linked to various genes governing bacterial blight and blast resistance in rice (Table 2) were analyzed for parental polymorphism between the donor parents and the recurrent parents so as to utilize in marker assisted selection for incorporating resistance to these disease into popular Basmati variety, Pusa Basmati 1 and the parental lines of the superfine grain aromatic rice hybrid Pusa RH10. Fig. 4 shows the polymorphism between the donor parents and the recurrent parent (Pusa Basmati 1) for markers linked to seven blast resistance genes.

Marker assisted breeding for bacterial blight resistance

One of the most successful cases in disease control is the use of multiple major genes for resistance to bacterial

blight caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Several genes have been associated with tightly linked DNA markers, and some of them have been cloned (*Xa1, xa5, xa13, Xa21, Xa26, Xa27*) and used for breeding BB resistant rice cultivars. The BB resistant rice cultivars released for commercial cultivation includes Angke (*Xa4+xa5*) and Conde (*Xa4+Xa7*) in Indonesia; NSIC Rc142 and NSIC Rc154 with the genes *Xa4+xa5+Xa21* in Philippines. Efforts were made for marker assisted transfer of BB resistance into a popular rice variety PR106 from Punjab [33] but did not yield commercially viable cultivars.

Marker assisted breeding for bacterial blight resistance in Basmati rice

Basmati from the Indian subcontinent is highly priced in the international market for its unique quality. The traditional Basmati cultivars are tall, prone to lodging, photoperiod and temperature sensitive, very low yielding and highly susceptible to Bacterial Blight (BB). In cases of severe infection, BB can cause up to 50% yield loss and severely affect the quality of the grains. BB is a serious constraint to Basmati rice production. There is no known source of BB resistance in the available aromatic rice germplasm. Thirty major genes governing BB resistance have been reported in the non-Basmati genotypes. As many as 14 of these genes have been mapped using molecular markers, which are being employed in marker assisted gene pyramiding for BB resistance [26, 34, 33].

A breeding strategy involving combined



(a) M-50 bp; Recurrent parent - Pusa Basmati 1, Donor parent - DHMAS70Q164-2a;

(b) M-50 bp; Recurrent parent - Pusa Basmati 1, Donor parent - IRBL75-CA;

- (c) M-50 bp; Recurrent parent Pusa Basmati 1, Donor parent IRBLB-b;
- (d) M-50 bp; Recurrent parent Pusa Basmati 1, Donor parent IRBL-9-w;

(e) M-50 bp; Recurrent parent - Pusa Basmati 1, Donor parent - IRBL5-M;

Fig. 4. Validation of molecular markers linked to seven blast resistance genes namely *Pi-1,Pi-Kh, Pita, Pi-z5, Pi-b, Pi-9* and *Pi-5* between donor parents and the recurrent parent Pusa Basmati - 1

phenotypic and molecular marker assisted selection was utilized for transferring two genes for bacterial blight resistance (*xa13* and *Xa21*) from non Basmati source (IRBB55) to Pusa Basmati 1, a popular semi-dwarf, high yielding Basmati rice variety [35].

- Firstly, marker assisted foreground selection was done for BB resistance genes *xa13* and *Xa21* using linked molecular markers namely RG136 and pTA248, respectively (Fig. 5).
- 2. Then, screening for grain and cooking quality characters namely aroma, L/B ratio and Kernel Elongation Ratio (KER) was carried out and
- Finally, background analysis of pyramided lines for recovery of recurrent parent genome using SSR markers spanning across the genome.

Background analysis enabled selection of recombinants with recurrent parent genome to the extent of 86.3% along with the quality traits. The extent of introgression of nonBasmati donor chromosome segments in the superior selections was estimated to be <7.8 Mb and <6.7 Mb in the *xa13* and *Xa21* linked genomic regions, respectively. The elite selection Pusa 1460-0132-6-7-67 with maximum genomic background



xa13: RG 136, a CAPS marker



Fig. 5. Marker assisted foreground selection in BC₁F₃ individuals from the cross Pusa Basmati 1/ IRBB55// Pusa Basmati 1 for the genes *xa13* and *Xa21*

and quality characteristics of the recurrent Basmati parent gave resistance reaction against BB, similar to that of the non-Basmati resistant check variety and recorded an yield advantage of 11.9% over the best check in the multilocation agronomic trial in the Basmati growing region of India. This line has been released as a new variety, Improved Pusa Basmati 1 for commercial cultivation in India, is an example of successful application of marker assisted selection to variety development [36].

MAS has been also successfully adopted for pyramiding three genes for bacterial blight resistance namely *xa5*, *xa13* and *Xa21* into a popular cultivar BPT5204 (Samba Mahsuri) and Improved Samba Mahsuri have been released for commercial cultivation in India during 2008 [37].

MAS for improving the parental lines of Pusa RH10 for BB resistance

Pusa RH10, the widely cultivated superfine grain aromatic rice hybrid and its parental lines Pusa6B and PRR78 are susceptible to bacterial blight disease. MAS has been adopted for transferring two genes *xa13* and *Xa21* for resistance to bacterial leaf blight in the parental lines (Pusa 6B and PRR78) of the superfine grain aromatic rice hybrid Pusa RH 10 [38].

Pusa1460, a Basmati rice variety was utilized as the donor for introgressing bacterial blight resistance genes xa13 and Xa21 into Pusa6B and PRR78 using marker assisted backcross breeding program. The markers RG136 and pTA248 linked to bacterial blight resistance genes xa13 and Xa21, respectively were used for foreground selection. 74 STMS markers polymorphic between Pusa6B and Pusa1460 and 54 STMS markers polymorphic between PRR78 and Pusa1460, were utilized for background selection to recover recurrent parent genome ranging from 85.14% to 97.30% and 87.04% to 92.81% in 10 best BC_2F_5 families of Pusa6B and PRR78, respectively. RM6100, a STMS marker linked to fertility restorer gene (Rf) was used for marker assisted selection of Rf gene in improved version of PRR78.

The extent of donor segments in the improved version of Pusa6B was estimated to be < 0.97 Mb and < 2.15 Mb in the genomic regions flanking *xa13* and *Xa21*, respectively whereas in improved PRR78, it was estimated to be < 2.07Mb and < 3.45Mb in the corresponding genomic regions. Improved lines of Pusa6B and PRR78 showed yield advantages up to 8.24% and 5.23%, respectively. The performance of the bacterial blight resistant version of Pusa RH10 produced by intercrossing the improved parental lines was on par or superior to the original Pusa RH10. The incorporation of bacterial blight resistance in the maintainer line will help in providing bacterial blight resistance in A X B plots also in addition to protection of the hybrid seed production plot (A X R).

Marker assisted breeding for Blast resistance in rice

Rice blast caused by the fungal pathogen, Magnaporthe grisea is a serious disease throughout the world. The dynamic evolution of the virulence in the blast fungus coupled with the epistatic action of different resistance genes in the host plant, makes breeding for blast resistance a constant challenge. Molecular genetics of blast resistance have been extensively studied, and many useful DNA markers corresponding to major genes conferring race-specific resistance have been identified [39]. Molecular breeding approach is being widely employed for the improvement of blast resistance in many high-yielding commercial rice cultivars. Of the 40 major blast resistance genes identified so far, about 30 genes have been mapped on different rice chromosomes, and tightly linked DNA markers have been developed. The use of DNA markers linked/ present within the gene or from the flanking region of the gene can improve the efficiency of MAS for rice

improvement. Additionally, several blast resistance genes could be combined using MAS in a single genetic background to develop rice cultivars with broadspectrum durable resistance to blast.

Marker assisted backcross breeding has been successfully utilized in transferring blast resistance genes Pi- K^h and Piz5 into the parental lines namely Pusa6B and PRR78 of the popular superfine grain aromatic rice hybrid Pusa RH10 A simultaneous but step wise transfer method was adopted for transferring the resistance genes Pi- K^h and Piz5 from the donor Tetep and C101A51, respectively. The improved versions of Pusa6B and PRR78 with two blast resistance genes Pi- K^h and Piz5 each have been developed through marker assisted backcross breeding at the Division of Genetics, IARI, New Delhi.

The science of genomics has an important contribution to make to the development of agriculture through MAS to help develop improved crop varieties. MAS is likely to be most effective when it is integrated with plant breeding and with a range of complementary disciplines [40]. With the emerging challenge of climate change there is a shift in the disease/ pest dynamics in different parts of India. The severe outbreak of brown plant hopper during Kharif 2008 in New Delhi is one of many examples where in the pest has caused havoc in its first year of occurrence. Therefore, the marker assisted breeding programme in rice which aims at pyramiding resistance genes against the most prevalent diseases of the region namely bacterial blight, blast as well as the emerging pest such as brown plant hopper into popular Basmati rice varieties has been initiated at IARI. With newer technologies through advances in the field of genomics, the challenge for plant breeders is to judiciously utilize these novels tools in molecular markerassisted breeding for developing commercially viable improved cultivars to address specific problems in different crops. This can be made possible only when MAS is integrated into traditional breeding practices, rather than being considered as a substitute.

References

- Eagles H., Bariana H., Ogbonnaya F., Rebetzke G., Hollamby G., Henry R., Henschke P. and Carter M. 2001. Implementation of markers in Australian wheat breeding. Australian Journal of Agricultural Research, 52: 1349–1356.
- Collard B. C. Y., Casiana M. Vera Cruz, McNally K. L., Virk P. S. and Mackill D. J. 2008. Rice molecular breeding laboratories in the genomics era: current status and future considerations. International Journal

of Plant Genomics, Article ID 524847, 25p. doi:10.1155/2008/524847.

- Sax K. 1923. The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics, 8: 552–560.
- Allen F. 1994. Usefulness of plant genome mapping to plant breeding. *In*: Gresshoff P. M. (ed.). Plant genome analysis. CRC Press, Boca Raton Florida, USA, pp 11–18.
- Tanksley S. D., Young N. D., Paterson A. H. and Bonierbale M. 1989. RFLP mapping in plant breeding: New tools for an old science. Biotechnology, 7: 257–264.
- Kolmer J. A. 2009. Genetics of Leaf Rust Resistance in the Soft Red Winter Wheat Caldwell. Crop Sci., 49: 1187-1192.
- Sears E. R. 1956. The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symposia in Biology, 9: 1-22.
- 8. McIntosh R. A., Yamazaki Y., Devos K. M., Dubcovsky J., Rogers W. J. and Appels R. 2003. Catalogue of Gene Symbols for Wheat. *In*: Tenth Intl. Wheat Genetics Symp. Proc., Instituto Sperimentale per la Cerealcoltura, Rome, Italy.
- McIntosh R. A., Yamazaki Y., Dubcovsky J., Rogers J., Morris C., Somers D. J., Appels R. and Devos K. M. 2008. Catalog of gene symbols for wheat. *In:* Eleventh Intl. Wheat Genetics Symp. Proc., 2008.
- Gupta S. K., Charpe A., Koul S., Prabhu K. V., Haq Q. M. R. 2005. Development and validation of molecular markers linked to an Aegilops umbellulataderived leaf-rust-resistance gene, *Lr9*, for markerassisted selection in bread wheat. Genome, **48**: 823-30.
- Gupta S. K., Charpe A., Prabhu K. V. and Haque Q. M. R. 2006. Identification and validation of molecular markers linked to the leaf rust resistance gene *Lr19* in wheat. Theor. Appl. Genet., DOI 10.1007/s00122-006-0362-7.
- Gupta S. K., Charpe A., Prabhu K. V. and Haque Q. M. R. 2006. Development and validation of SCAR markers co-segregating with an *Agropyron elongatum* derived leaf rust resistance gene *Lr24* in wheat. Euphytica, **150**: 233-240.
- Cherukuri D. P., Gupta S. K., Charpe A., Koul S. Prabhu K. V., Singh R. B. and Haq Q. M. R. 2005. Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. Euphytica, 143:19-26.
- Samsampour D., Maleki Zanjani B., Singh A., Pallavi J. K. and Prabhu K. V. 2009. Marker assisted selection to pyramid seedling resistance gene *Lr24* and adult plant resistance gene *Lr48* for leaf rust resistance in wheat. Indian J. Genet. Plant Breed., 69: 1-9.

- Todorovska E., Christov N., Slavov S., Christova P. and Vassilev D. 2009. Biotic Stress Resistance in Wheat – Breeding and genomic selection implications. Biotechnol. & Biotechnol. Eq., 23: 1417-1426.
- Singh R. P., Nakamura K. and Huerta-Espino, J. 2001. Leaf rust resistance genes in japanese wheat cultivars. Breeding Sci., 51: 83-87.
- 17. **Pretorius Z. A. and Le Roux J.** 1988. Occurrence and pathogenecity of *Puccinia recondita* f. sp. *tritici* in South Africa during 1986 and 1987. Phytophylactica, **22**: 225-228.
- Wamishe Y. A. and Milus E. A. 2004. Genes for adultplant resistance to leaf rust in soft red winter wheat. Plant Dis., 88: 1107-1114.
- McCouch S. R., Kochert G., Yu Z. H., Wang Z. Y., Khush G. S., Coffman R. W. and Tanksley S. D. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet., 76: 815-829.
- McCouch S. R., Chen X., Panaud O., Temnykh S., Xu Y., Cho Y. G., Huang N., Ishii T. and Blair M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol. Biol., 35: 89-99.
- Mackill D. J., Nguyen H. T. and Zhang J. 1999. Use of molecular markers in plant improvement programs for rainfed lowland rice. Field Crops Research, 64: 177-185.
- Jena K. K. and Mackill D. J. 2008 Molecular markers and their use in marker-assisted selection in rice. Crop Science, 48: 1266–1276.
- McCouch S. R., Teytelman L., Xu Y., Labos K. B., Clare K. and Walton M. 2002. Development and mapping of 2240 new SSR markers in rice (*Oryza* sativa L.). DNA Res., 9: 199 – 207.
- McNally K. L., Bruskiewich R., Mackill D., Buell C. R., Leach J. E. and Leung H. 2006. Sequencing Multiple and Diverse Rice Varieties. Connecting Whole-Genome Variation with Phenotypes. Plant Physiol., 141: 26-31.
- Dai L. Y., Liu X. L., Xiao Y. H. and Wang G. L. 2007. Recent advances in cloning and characterization of disease resistance genes in rice. J. Integr. Plant Biol., 49: 112–119.
- Huang N., Angeles E. R., Domingo J., Magpantay G., Singh S., Zhang G., Kumaravadivel N., Bennett J. and Khush G. S. 1997. Pyramiding of bacterial blight resistance genes in rice: marker assisted selection using RFLP and PCR. Theor Appl Genet, 95: 313–320.
- Fjellstrom R., McClung A. M., Shank A. R. 2006. SSR markers closely linked to the *Pi-z* locus are useful for selection of blast resistance in a broad array of rice germplasm. Mol. Breed. 17:149–157.

- Sharma T. R., Shanker P., Singh B. K., Jana T. K., Madhav M. S., Gaikwad K., Singh N. K., Plaha P. and Rathour R. 2005. Molecular mapping of rice blast resistance gene *Pi-kh* in the rice variety. Tetep. J. Plant Biochem. Biotechnol, 14:127-133.
- Fuentes J. L., José Correa-Victoria F., Escobar F., Prado G., Aricapa G., Duque M. C., Tohme J. 2007. Identification of microsatellite markers linked to the blast resistance gene *Pi-1(t)* in rice. Euphytica, 160: 295–304.
- Eizenga G. C., Agrama H. A., Lee F. N., Yan W. G. and Jia Y. 2006. Identifying novel resistance genes in newly introduced blast resistant rice germplasm. Crop Sci., 46: 1870–1878.
- Hayashi K., Yoshida H., Ashikawa I. 2006. Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance genes. Theor. Appl. Genet., 113: 251–260.
- Jeon J. S., Chen D., Yi G. H., Wang G. L. and Ronald P. C. 2003. Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast. Molecular Genetics and Genomics, 269: 280-289.
- Singh S., Sidhu J. S., Huang N., Vikal Y., Li Z., Brar D. S., Dhaliwal H. S. and Khush G. S. 2001. Pyramiding three bacterial blight resistance genes (*Xa5, Xa13, Xa21*) using marker assisted selection into indica rice cultivar PR106. Theor. Appl. Genet., 102: 1011–1015.
- Sanchez A. C., Brar D. S., Huang N., Li Z. and Khush G. S. 2000. Sequence Tagged Site markerassisted selection for three bacterial blight resistance genes in rice. Crop Sci., 40: 792–797.
- Joseph M., Gopalakrishnan S., Sharma R. K., Singh A. K., Singh V. P., Singh N. K. and Mohapatra T. 2004. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. Molecular Breeding, 13: 377-387.

- Gopalakrishnan S., Sharma R. K., Rajkumar K. A., Joseph M., Singh V. P., Singh A. K., Bhat K. V., Singh N. K. and Mohapatra T. 2007. Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. Plant Breeding, 127: 131-139.
- Sundaram R. M., Vishnupriya M. R., Biradar S. K., Laha G. S., Reddy G. A., Shobha Rani N., Sarma N. P. and Sonti R. V. (2008) Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. Euphytica, 160: 411–422.
- Basavaraj S. H., Singh V. K., Singh A., Singh A., Singh A., Yadav S., Ellur R. K., Singh D., Gopala Krishnan S., Nagarajan M., Mohapatra T., Prabhu K. V. and Singh A. K. 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Molecular Breeding, DOI 10.1007/s11032-010-9407-3.
- Fjellstrom R., Conaway-Bormans C. A., McClung A. M., Marchetti M. A., Shank A. R., Park W. D. 2004. Development of DNA markers suitable for markerassisted selection of three *Pi* genes conferring resistance to multiple *Pyricularia grisea* pathotypes. Crop Sci., 44: 1790–1798.
- 40. Reece D. J. and Haribabu E. 2007. Genes to feed the world: The weakest link? Food Policy, **32**: 459–479.